

Gram-positive organisms in endodontic infections

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Culture-based studies in endodontics have more or less overlooked the significance of Gram-positive facultative bacteria in recent decades. By contrast, Gram-negative anaerobes have been extensively studied because of their frequent recovery in primary root canal infections and their association with acute manifestations of apical periodontitis. More recent years have seen a renewed interest in Gram-positive facultatives as these organisms are common in samples from root-filled teeth affected by apical periodontitis. Structural components of the robust bacterial cell wall of Gram positives protect them from noxious environmental factors. Additionally, the majority of these organisms express fast-adaptive properties when exposed to extreme conditions, thus making them potentially interesting as causal elements in post-treatment endodontic disease. This review relates to different aspects of Gram-positive bacteria and their adaptive responses when being exposed to stressful conditions such as endodontic treatment procedures.

Introduction

Microbiological sampling of infected root canals in the early 20th century frequently recovered Gram-positive facultatives. As a result, these fast-growing organisms were considered major etiologic elements in endodontic infections (1–3). With the advent of improved methods for anaerobic cultivation, by the end of the 1960s, difficult-to-culture organisms with high demands for growth under anaerobic conditions were recognized (4) and were found to predominate in primary root canal infections (5–10). In the years that followed, attention was drawn to their pathogenic potential and the participation of black-pigmented Gram negatives in acute presentations of apical periodontitis was confirmed (11, 12). But a whole range of other Gram negatives were also identified as potential pathogens in endodontic infections (13, 14). Concomitantly, Gram-positive facultatives became of less interest because of their recovery in small numbers in primary endodontic infections. Even their clinical significance was questioned as they were assumed to represent contamination during the sampling procedure (15, 16).

Because of their frequent recovery in previously root-filled teeth (4, 17–21) and from teeth undergoing root canal treatment (22–25), a renewed interest in Gram-positive bacteria has occurred in recent years. Especially

bacteria belonging to the enterococcus group have attracted considerable attention, most notably *Enterococcus faecalis*, as these are frequent isolates in culture positive samples taken from filled root canals (4, 17–21). The fact that sampled teeth were affected by apical periodontitis suggested the involvement of these organisms in post-treatment endodontic disease. Also, other facultatives may be of interest. Sampling teeth with signs of apical periodontitis refractory to treatment, Chávez de Paz et al. (25) reported frequent isolation of lactobacilli and streptococci, while anaerobes were conspicuous by their rare occurrence. Collectively, these findings suggest that, in case total eradication has failed, endodontic treatment may select for the most robust segment of the root canal microbiota viz. facultative anaerobes organisms that may be responsible for post-treatment apical periodontitis (see also Table 1).

Of distinct clinical interest in this context are the mechanisms that afford consortia of organisms to prevail in the root canal environment in spite of rigorous anti-microbial efforts in root canal therapy and the resulting limited nutrient availability. In general, adaptive features of bacterial organisms are built on diverse mechanisms of stress responses that will vary in speed and intensity depending on innate physiological resources. Moreover, expression of adap-

Table 1. Data from different studies screening bacteria prevailing after instrumentation and intracanal dressings

	Byström and Sundqvist. (22, 148)			Gomes et al. (23)	Peters et al. (24)	Chávez de Paz et al. (25)	
Number of cases	7	8	6	31	15	74	33
Evidence of apical periodontitis	Yes	Yes	Yes	Variable	Yes	Yes	Yes
Irrigant used	Saline	0.5% NaOCl	5% NaOCl	2.5% NaOCl	2% NaOCl	0.5% NaOCl	0.5% NaOCl
Intracanal dressing	None	None	None	None	Ca(OH) ₂	Ca(OH) ₂	Ca(OH) ₂ , 5% IKI
Gram-positive cocci							
Coagulase negative <i>Staphylococci</i>	0	0	0	1	1	9	8
<i>Enterococcus</i> spp.	0	0	0	7	0	26	0
<i>Gemella</i> spp.	0	0	0	2	1	0	0
<i>Peptostreptococcus</i> spp.	7	1	1	11	2	7	2
<i>Streptococcus</i> spp.	6	3	2	46	1	38	7
Gram-positive rods							
<i>Actinomyces</i> spp.	0	0	0	6	3	9	3
<i>Bifidobacterium</i> spp.	0	0	0	0	2	9	9
<i>Clostridium</i> spp.	0	0	0	1	0	2	2
<i>Eubacterium</i> spp.	5	3	2	4	1	3	3
<i>Lactobacillus</i> spp.	3	2	2	8	0	40	9
<i>Propionibacterium</i> spp.	0	1	0	6	3	13	2
Gram-negative cocci							
<i>Veillonella</i> spp.	0	0	0	3	2	5	1
Gram-negative rods							
<i>Bacteroides</i> spp.	3	2	1	1	1	0	0
<i>Campylobacter rectus</i>	0	0	1	0	0	0	0
<i>Capnocytophaga</i> spp.	0	0	1	1	2	0	0
<i>Enterobacteria</i> (lactose positive)	1	1	0	0	0	3	4
<i>Fusobacterium</i> spp.	3	6	3	5	3	7	2
<i>Haemophilus</i> spp.	0	0	0	1	0	0	0
<i>Porphyromonas</i> spp.	0	2	0	1	0	1	0
<i>Prevotella</i> spp.	2	2	1	11	1	5	6
Total isolates (strains per case)	30 (4.3)	22 (2.8)	14 (2.3)	115 (3.7)	23 (1.5)	177 (2.4)	58 (1.8)

Figures indicate number of strains isolated. Most frequently isolated in bold.

tive mechanisms is increased when organisms establish themselves within micro-communities (community theory), making it possible for fast-adaptive bacteria to reproduce and initiate formation of biofilms (26). In biofilms, even relatively more susceptible organisms are capable of surviving and may potentially play a role in endodontic treatment failures. This review focuses on different aspects on Gram-positive organisms in endodontic infections, describes frequently recovered species, and the potential adaptive responses that these organisms may express when the conditions in the root canal environment change.

Bacterial species in post-treatment cases

Cross-sectional, culture-based studies have given important clues about the variety of bacterial organisms that may colonize root canals of teeth where the pulp

has become necrotic (5–10). Clearly, anaerobes predominate and may make up 97% of the cultivable flora in teeth where the pulp chamber has been without direct communication with the oral cavity. Culture studies from treated teeth, on the other hand, have revealed a much higher proportion of facultatives (4, 17–21). These studies also often isolated species in monocultures.

The reduction of Gram-negative organisms following endodontic treatment and the subsequent proportional increase of Gram-positives facultatives (see Fig. 1) give support for the view that anti-microbial treatment measures in endodontics are more efficient against obligate anaerobes and less so towards a whole set of facultatives. This supposition, of course, takes into account that all these organisms indeed were present at the outset and were not latecomers from the oral environment. But even so, contaminants, once becoming established in the root canal system of teeth, may

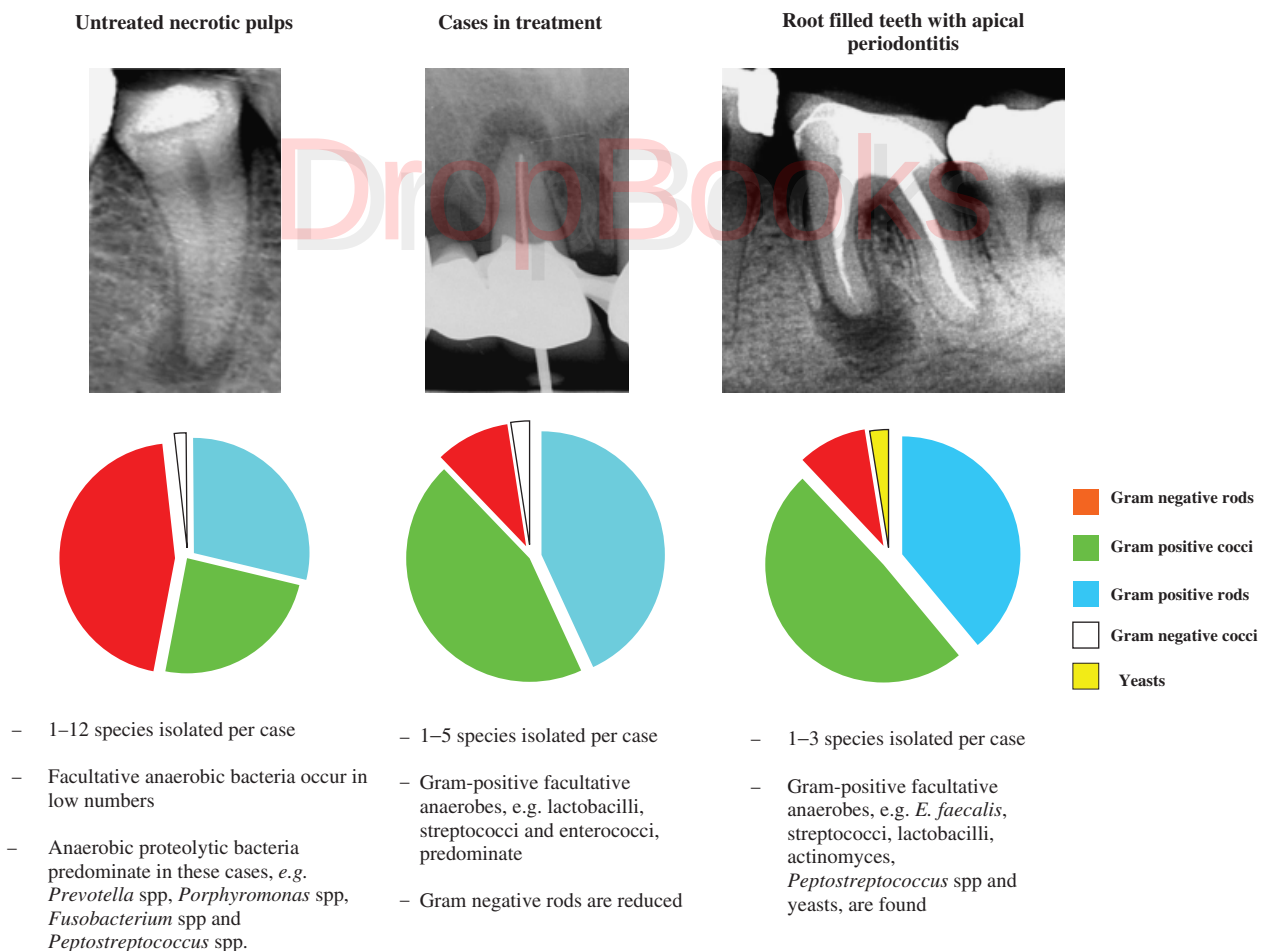


Fig. 1. Pie charts showing the proportion of organisms isolated in studies of untreated necrotic pulps (5–10), cases undergoing treatment (4, 22–25, 30, 31), and root-filled teeth with apical periodontitis (4, 17–21, 68).

exert pathogenic effects and should therefore not be excluded as potential causal elements in post-treatment endodontic disease.

Streptococci

Streptococci normally inhabit the oral cavity and are early colonizers of tooth surfaces upon dental plaque formation. Their role in endodontic infections, however, has not yet been ascertained although they are prevalent culture isolates. Yet, these organisms possess outstanding abilities to penetrate dentinal tubules both individually and in co-aggregation with other organisms (see the paper by Robert Love in this issue (27). Furthermore, streptococci have been identified in deep carious lesions (28, 29). In addition, oral streptococci show potential adaptive responses to extreme environmental change that may have implications for their potential to survive root canal treatment.

A variety of streptococcal species have been isolated at significant rates in studies using rigorous measures to control for inclusion of contaminating organisms, e.g. in samples from primary infected teeth (4, 5, 8), during root canal treatment (23, 25), and in retreatment cases (4, 17, 18). Applying a combination of biochemically based methods, 93% of the streptococci, isolated after instrumentation and application of intra-canal dressings, were identified to species level (30). The majority of streptococcal species belonged to the *Streptococcus mitis* and *S. anginosus* groups of which *S. gordonii*, *S. anginosus*, and *S. oralis* were the most prevalent (25, 30, 31), see Table 2.

S. gordonii is an early colonizer in dental plaque. Although no evidence exists regarding its pathogenicity in apical periodontitis, *S. gordonii* has interesting capabilities that give indications of its pathogenic potential. This organism promotes co-adhesion of *Porphyromonas gingivalis* to dental plaque (32–34). *S. gordonii* also promotes *in vitro* invasion of dentinal tubules of *P. gingivalis* (35). Furthermore, the intracellular transport of manganese in *S. gordonii* has been found to be significant for the formation of biofilms (36, 37).

S. anginosus belongs to the *S. anginosus* group (formerly *S. milleri*). This organism has become known as an important pathogen in respiratory infections, sub-acute bacterial endocarditis, and upper digestive tract cancer (38–42). Its pathogenic capacity is shared with

other members of the *S. anginosus* group including *S. intermedius* and *S. constellatus*. In the oral cavity, *S. anginosus* is considered a regular commensal frequently found in deep periodontal pockets and abscesses (43). Furthermore, *S. anginosus* owns mechanisms for attachment and co-aggregation with other bacteria, a property that fits well with its ability to become established within micro-communities. Along with other streptococci, this feature gives indication of a potential pathogenic role in persistent endodontic infections.

S. oralis is a member of the mitis group of the viridans streptococci (44). This organism is a major contributor to dental plaque and has been widely studied for its ability to adapt to acidic environments (45–47). In this regard, different surface-associated proteins seem to play important regulating and adaptive roles (47). Although the significance of *S. oralis* in root canal infections has not been well established, such physiological mechanisms may be significant for its survival power.

The ability of streptococci to initiate biofilm formations can be explained by their release of different extra-cellular proteins and by their production of fimbriae. By virtue of fimbriae, *S. parasanguis* has the ability to attach, colonize, and thrive in environments of fluxes in pH, temperature, mechanical stress, and nutrient availability in the oral cavity (48–50). These features give this organism, in addition, outstanding capabilities to spreading, when introduced to the bloodstream, and attaching to predisposing heart valves in endocarditis-prone individuals (48). Yet, as with other streptococci, research is needed to assess the precise role of *S. parasanguis* in root canal infections.

By contrast, polysaccharide-producing species such as *S. salivarius*, *S. sanguis*, and *S. mutans* are rarely seen in the persisting root canal flora. This is likely to be explained by the non-favorable environment that the root canal offers these organisms, while they are predominant in saliva (*S. salivarius*) and plaque (*S. sanguis* and *S. mutans*). It is certainly possible that they can appear in the most coronal portion of root canals in conjunction with penetrating caries. It is not unreasonable to believe that species like *S. mutans* also can be carried into root canals by contamination of plaque or saliva during endodontic treatments. In any case, the rare occurrence of polysaccharide-producing streptococci and the rather frequent occurrence of other

Table 2. Isolation frequency of different bacterial species in consecutive root canal samples (RCS) in studies by Chávez de Paz et al (25, 30, 31)

	RCS 1 (183 cases)					RCS 2 (78 cases)					RCS 3 (11 cases)							
	Isolation frequency					Isolation frequency					Isolation frequency							
	+	++	+++	++++		+	++	+++	++++		+	++	+++		+	++	+++	
Gram-positive cocci																		
Coagulase negative <i>Staphylococci</i>	19	3	5	10	1	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Enterococcus faecalis</i>	46	7	18	8	13	18	9*	7	2	2	2	2	2	2	2	2	2	2*
<i>Peptostreptococcus</i> spp.	15	3	8	2	2	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Streptococcus anginosus</i>	21	3	6	9	3	3	1	1	1	1	1	1	1	1	-	-	-	-
<i>S. gordonii</i>	34	5	14	10	5	7	5	2	-	1	1	1	1	1	1	1	1	1
<i>S. intermedius</i>	6	-	2	2	2	2	-	1	1	1	1	1	1	1	-	-	-	-
<i>S. mutans</i>	5	1	3	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>S. oralis</i>	20	2	8	8	2	8	7	1	-	-	-	-	-	-	-	-	-	-
<i>S. parasanguis</i>	6	-	1	2	3	3	2	1	-	-	-	-	-	-	-	-	-	-
<i>Streptococcus</i> spp.	8	1	4	3	-	1	-	1*	-	-	-	-	-	-	-	-	-	-
Gram-positive rods																		
<i>Actinomyces israelii</i>	2	1	1	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-
<i>A. meyerii</i>	7	-	1	4	2	1	-	1	-	-	-	-	-	-	-	-	-	-
<i>A. naeslundii</i>	3	1	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>A. odontolyticus</i>	7	-	3	2	2	3	2	1	-	1	-	1	-	1	1	1	1	1
<i>Actinomyces</i> spp.	1	-	1	-	-	2	-	1*	-	1*	-	1*	-	-	-	-	-	-
<i>Bifidobacterium breve</i>	3	-	1	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>B. dentium</i>	11	3	2	3	3	3	3	-	-	-	-	-	-	-	-	-	-	-
<i>B. longum</i>	6	-	4	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 2. (Continued)

	RCS 1 (183 cases)					RCS 2 (78 cases)					RCS 3 (11 cases)				
	Isolation frequency	+	++	+++	++++	Isolation frequency	+	++	+++	++++	Isolation frequency	+	++	+++	+
<i>Bifidobacterium</i> spp.	2	-	1	1	-	-	-	-	-	-	-	-	-	-	-
<i>Clostridium</i> spp.	4	2	2	-	-	1	1	-	-	-	-	-	-	-	-
<i>Enterobacterium limosum</i>	3	1	1	1	-	-	-	-	-	-	-	-	-	-	-
<i>E. nodatum</i>	7	2	3	1	1	1	1*	-	-	-	-	-	-	-	-
<i>Enterobacterium</i> spp.	1	1	-	-	-	1	1*	-	-	-	-	-	-	-	-
<i>L. acidophilus</i>	4	-	2	2	-	1	1	-	-	-	-	-	-	-	-
<i>L. casei</i>	11	2	7	-	2	2	-	2*	-	-	-	-	-	-	-
<i>L. crispatus</i>	8	-	6	1	1	1	1	-	-	-	-	-	-	-	-
<i>L. curvata</i>	2	-	1	1	-	-	-	-	-	-	-	-	-	-	-
<i>L. delbrueckii</i>	10	2	4	3	1	2	1	1	-	-	-	-	-	-	-
<i>L. paracasei</i>	22	1	6	7	8	6	-	5	1	1	1	1	1	1	1
<i>L. plantarum</i>	3	-	1	1	1	2	-	1	1	-	-	-	-	-	-
<i>L. rhamnosus</i>	4	-	2	2	-	1	1	-	-	-	-	-	-	-	-
<i>L. salivarius</i>	3	2	1	-	-	-	-	-	-	-	-	-	-	-	-
<i>Lactobacillus</i> spp.	1	-	-	1	-	-	-	-	-	-	1	1	-	1*	1
<i>Olsenella uli</i> (<i>L. uli</i>)	29	2	12	7	8	8	4	3	1	2	2	2	2	2	2
<i>Propionibacterium acnes</i>	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-
<i>P. propionicum</i>	18	3	6	3	6	5	2	1	2	1	1	1	1	1	1
<i>Propionibacterium</i> spp.	1	-	-	1	-	2	1	1	-	-	2	2	2	2*	2*

Gram-negative cocci

Table 2. (Continued)

	RCS 1 (183 cases)					RCS 2 (78 cases)					RCS 3 (11 cases)				
	Isolation frequency	+	++	+++	++++	Isolation frequency	+	++	+++	++++	Isolation frequency	+	++	+++	+
<i>Veillonella</i> spp.	10	4	4	1	1	-	-	-	-	-	-	-	-	-	-
Gram-negative rods															
<i>Enterobacteria</i> (lactose positive)	11	1	6	2	2	1	1	-	-	-	-	-	-	-	-
<i>Fusobacterium</i> spp.	11	3	5	2	1	-	-	-	-	-	-	-	-	-	-
<i>Porphyromonas</i> spp.	1	-	1	-	-	-	-	-	-	-	-	-	-	-	-
<i>Prevotella</i> spp.	16	1	13	2	-	-	-	-	-	-	-	-	-	-	-
Yeasts															
<i>Candida</i> spp.	2	1	1	-	-	-	-	-	-	-	-	-	-	-	-
Total	405	58	168	109	70	86	45	31	10	11	11	11	11	11	11

A total of 183 teeth undergoing treatment with persisting signs of apical periodontitis were analyzed. Number of isolates was considerably reduced at subsequent appointments.

*One of these strains was not isolated in previous sample.

streptococcal species make a strong case for the need for identifying isolated bacteria to species level.

Lactobacilli

Lactobacilli are Gram-positive non-spore-forming rods with complex nutritional requirements. They are strictly fermentative, and grow well in acidic environments and use glucose as a major source for carbon to produce lactic acid, CO₂, ethanol, and acetic acid. Lactobacilli are ubiquitous and widespread commensal bacteria in the human and animal micro-flora. In humans they are normal residents of the gut, oral cavity, and vagina (51, 52). The majority of these organisms are used as adjuvants against gastrointestinal disorders or dietary supplements (probiotics), as well as biological food processors because of their high fermentative properties (53, 54). By virtue of metabolic production of lactate and short-chain fatty acids, lactobacilli have well-defined and proven clinical effects for the treatment and/or prevention of diseases of intestinal origin (55). Furthermore, lactobacilli are considered to be of low pathogenicity. By contrast, these organisms have antagonistic effects towards other microbial pathogens in the human gut (56), and severe *Lactobacillus* infections may occur in immuno-compromised patients (53).

Culture-growing lactobacilli are seldom reported to species level most likely because of lack of a universal protocol for their identification. Commercially available carbohydrate fermentation tests fail to identify various lactobacillus species (57). However, highly standardized whole-cell protein patterns obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis have been proven useful (31, 58, 59). Other identification methods include molecular-based analyses including PCR, RAPD-PCR, and the system MicroSeq 500 16S rDNA (60, 61).

In endodontic infections, the relevance of lactobacilli is not well defined and they have often been regarded as transient contaminants (4, 15). Yet, these organisms are common in deep caries lesions (28, 29, 62), in root canals of teeth undergoing root canal treatment (25, 31) (see also Table 2), and in root-filled teeth associated with apical periodontitis (17, 18). Thus, it is not unreasonable to assume that lactobacilli with their high resistance to environmental changes are capable of growing and multiplying in root canals. In the study by

Chávez de Paz et al. (31), the most frequently recovered species were *L. uli* and *L. paracasei*. *L. uli* has recently been re-classified to *Olsenella uli* on the basis of phenotypic characteristics and 16S rRNA sequence analysis (63). This organism has been isolated from gingival crevices and periodontal pockets from healthy and diseased patients (64). Information is scarce regarding its pathogenic capacity in human infections. *O. uli* produces large quantities of lactic acid and may resist the exposure to alkaline pH environments (Chávez de Paz et al., unpublished data). This organism, recovered from gingival pockets, has been shown to produce large quantities of lactic acid, which may impact the periapical inflammatory process (64).

Also *L. paracasei* was frequently isolated in the study by Chávez de Paz et al. (31). This is a normal resident of the human intestine and a transient inhabitant of the oral cavity (64). In human infections, *L. paracasei* has been found to be involved in bacteremias of hospitalized patients with a depressed immune status (66). Recently, *L. paracasei* was the sole etiological agent of a life-threatening intravascular infection (67). Although the pathogenic implications of *L. paracasei* in endodontic infections are unknown, it carries efficient adaptive capabilities to resist extreme environments, e.g. alkaline pH (Chávez de Paz et al., unpublished data).

Species like *L. acidophilus* and *L. salivarius* are normal inhabitants of plaque and caries lesions but are not frequently identified in root canal infections.

Enterococci

The resilient nature of *E. faecalis* in endodontic infections is well documented (4, 17–21, 68). In many studies, *E. faecalis* has been reported to occur in monocultures. By contrast, this organism and other genera of enterococci are infrequent in primary root canal infections, raising questions regarding their origin in post-treatment endodontic cases. Plausible explanations include unintentional inclusion during root canal treatment, or by leaving the root canal space open to the oral environment (69) as well as post-treatment leakage along the margins of permanent restorations. In any case it seems that once established in the root canal system, enterococci are able to resist a variety of endodontic treatment efforts for its eradication. For instance, *E. faecalis* has been found to prevail

after intra-canal dressings with Ca (OH)₂ (70, 71), clindamycin and 5% IKI (69, 72–75), tetracycline, and erythromycin (76). In serial sampling of teeth refractory to treatment, enterococci have been shown to prevail as a frequent isolate (25, 77).

The pathogenic potential of *E. faecalis* has yet to be confirmed. In Fabricius thesis (78), *E. faecalis* was inoculated as a monoculture in nine teeth with devitalized pulps. Although surviving for 6 months only weak inflammatory periapical responses were provoked (79). This study nevertheless showed that *E. faecalis* holds strong innate adaptive mechanism for survival within the confines of the root canal system (80). Recently, Kayaoglu and Ørstavik (81) reviewed the potential virulence factors of *E. faecalis*. Briefly, these factors include aggregation substance, surface adhesions, sex pheromones (see review by Sedgley and Clewell in this issue (82), extra-cellular superoxide production, gelatinase, and toxic cytolysin.

In vitro assessments on the resilient nature of *E. faecalis* to endodontic anti-microbials are replete (74, 83–91). From these studies, important mechanisms for this organism to circumvent anti-microbial effects have been revealed. For instance, the innate alkalo-tolerant characteristic of *E. faecalis* is clinically related with reduced susceptibility to calcium hydroxide intra-canal dressings.

Undoubtedly, *E. faecalis* is a most interesting organism in post-treatment endodontic infections given its innate and acquired adaptive properties. Yet, it must not be forgotten that endodontic infections most often are polymicrobial in nature. Isolation of only one species may, therefore, not necessarily be an indication of mono-infection, but of the possibility that other organisms present did not reach detection level. For example, in the Molander et al. (17) study in one-third of the 100 retreatment cases with apical periodontitis, samples were negative suggesting that the sampling techniques used for the detection of cultivable species was not invariably optimal. Further efforts should be directed to elucidate the role of enterococci in endodontic infections.

Other Gram-positive rods

Actinomyces spp. belong to the primary colonizers of clean tooth surfaces and are relatively frequent isolates in endodontic infections (92, 93). The fimbriae on the cell surface of these organisms are important for its

virulence and its establishment in extra-radicular endodontic infections (apical actinomycosis) (94). On a species level, *A. israelii* and *A. meyerii* have been specifically implicated (95–97). Recently, a new species *A. radidentis* has been identified in pure cultures from root canals of teeth with periapical lesion persistence (98). Actinomyces may also survive in nutritionally deprived environments by expressing extra-cellular enzymes that allow the organism to metabolize sucrose and urea (99, 100).

Propionibacterium propionicum is a facultative anaerobic organism formerly known as *Arachnia propionica* (101). This bacterium is a normal resident of the oral cavity and has been repeatedly found in persisting intra-radicular and extra-radicular endodontic infections that do not respond to conventional endodontic treatment (102). Although its pathogenic capacity still remains unclear (103), it seems that *P. propionicum* shares similar invasive characteristics as actinomyces (for a review, see reference (104)).

Bifidobacteria present characteristics similar to lactobacilli. These organisms are normal inhabitants of the human gut and are also commonly used as probiotic bacteria. Most of the species colonizing the oral cavity are transient colonizers (52). However, *B. dentium* is one of the few species of this genus that inhabits the oral cavity and resides in deep periodontal pockets (105). Members of this genus have also been found after anti-microbial root canal treatment (24, 31). Yet, similar to lactobacilli, no knowledge exists as to its pathogenic capacity in apical periodontitis.

Eubacteria are asaccharolytic micro-organisms implicated in marginal periodontitis, advanced caries, and dento-alveolar abscesses (106). This genus has not been further linked to endodontic infections, although species like *E. nodatum*, *E. alactolyticum* (re-classified as *Pseudoramibacter alactolyticus*) (107) were frequently reported in early studies (4, 8). This lack of information may be associated with the difficulty in growing these organisms under laboratory conditions (108).

In conclusion, a variety of both facultative and strict anaerobic Gram-positive cocci and rods have been recovered from post-treatment endodontic cases. A common denominator for the persistence of these organisms is that, contrary to several Gram-negative anaerobes, little is known about their pathogenic potentials and the extent to which they are contributory to post-treatment endodontic lesions in optimally treated cases.

Adaptive mechanisms

For survival and growth, bacterial organisms that may have survived the endodontic treatment procedures, including mechanical preparation and use of chemicals for disinfection by irrigation and dressing, must find ways to adapt to the new conditions in the root canal. In particular, this means adjustment to changed nutritional supplies. These are likely to be scarce unless the root canal filling has failed to block the portals of entry to the root canal space along which nutritional elements may be transported. They include the main apical foramen and accessory canals as well as the oral environment and associated leakage potentials along margins of coronal restorations. The extent to which the root filling can block these entries is crucial. Coronal leakage is easily controllable, whereas blocks of the main apical foramen and accessory canals may not invariably be efficient. An important variable in this context is the extent to which the apical foramen was overprepared and laterally transported (see a review by Bergenholtz & Spångberg (109)). If large, substantial amounts of tissue fluid and inflammatory exudates may percolate and continue to support growth of any organism with proteolytic capacity. Often, however, nutritional supply will become very limited. Therefore, only organisms that have a capacity to adapt and find subsistence under such conditions will prevail. Many of the Gram positives that are in suppressed numbers in primary root canal infections make use of various adaptive mechanisms, which may explain their proportional increase relative to Gram-negative anaerobes in post-treatment cases.

Location

The character of the micro-flora is likely to be determined by the location in that, near canal exits to the periapical tissue environment, nutrient availability is likely to be richer than at a far distance. Given that root fillings seldom hermetically seal the root canal space (110), sites that were not properly instrumented for example non-instrumented fins and crevices, voids in hard tissue repair processes and areas of resorption along the root canal wall (see Fig. 2) may serve as spots for bacterial survival and growth. In most cases, these locations cannot be ascertained clinically or radiographically, and represent distinct challenges in endodontic therapy (111).

Environmental stress factors in infected root canals

Nutrients in short supply initiate starvation-stress responses. By interacting with other bacteria, sources for amino acids and vitamins are obtained (112). Furthermore, in starvation, bacteria modify their nutritional demands (113). Bacteria will then limit the amount of nutrients, they require, in order to save the energy used for metabolism, thus enabling them survival for long periods of time. For instance, streptococci under stressful conditions will utilize their synthesized exo-polysaccharides, a feature not observed under normal circumstances (114).

Alkaline ions have lethal effects on bacteria by destroying cell membranes and protein structures (115). When calcium hydroxide is applied as intra-canal dressing in root canals, an overall alkalization of the root canal environment occurs. This alkaline shift is not necessarily homogenous along the length of the root canal (116, 117) and higher pH readings are registered at cervical portions and the lowest in the apical region (118).

Bacteria able to sustain high alkaline levels can be divided into two groups: alkalophilic and alkalo-tolerant species. Both kinds of micro-organisms may grow even above pH 10, but alkalophiles are unable to sustain neutral pH as their optimal growth condition is around pH 9. This is the case for *Bacillus* spp. for example. On the other hand, alkalo-tolerant organisms grow optimally around neutrality, e.g. enterococci (119).

It is intriguing that organisms growing in aciduric environments like streptococci and lactobacilli prevail in root canals after calcium hydroxide dressings (25), suggesting capability to adapt to alkaline shifts. This capacity relies on mechanisms to maintain a homeostasis between external and intra-cellular pH (119). To achieve this balance, adaptation includes synthesis of extra-cellular proteins (120, 121) and the utilization of certain intrinsic mechanisms such as the proton-pump (122). The proton-pump allows transport of cations and protons into the cell body to keep the cytoplasmic pH neutral (123–125). *E. faecalis* utilizes such a mechanism (122).

In a biofilm community, survival and means of adaptation to extreme pH levels could be more achievable (114). As demonstrated in Fig. 3, a community of seven bacterial species including *E.*

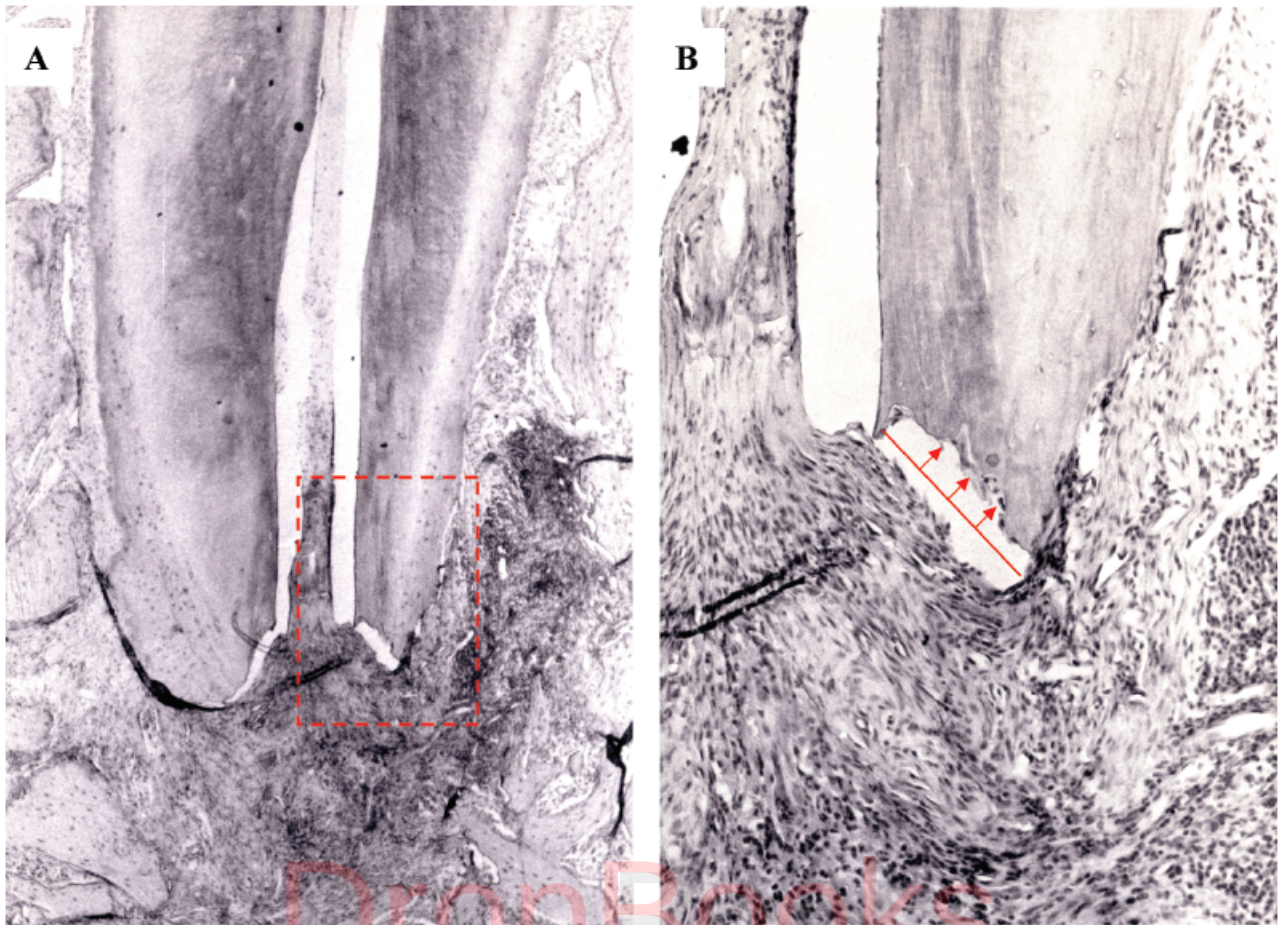


Fig. 2. Apical region of a monkey's tooth showing root resorption, where bacteria may be located (dotted square) (A). Magnification of the area is seen in (B). Specimen is unpublished data obtained from the thesis by Fabricius (78) Courtesy of Professor Gunnar Dahlén.

faecalis, *F. nucleatum*, *L. paracasei*, *O. uli*, *S. anginosus*, *S. gordonii*, and *S. oralis* formed clusters when exposed to an alkalinized environment (Chávez de Paz et al., unpublished data). This finding suggests that under alkaline shifts, bacteria will co-aggregate to meet their individual needs for survival.

Oxidative stress is an important factor that will influence bacterial selection in infected root canals (see a review by Sundqvist and Figdor (126)). This factor implies the release of noxious by-products formed during the metabolic pathway of oxygen. Bacteria exposed to such environmental conditions need to find ways to adapt (127–129). In a closed system such as the root canal the oxygen tension tends to be low. Hence, the growth of strict anaerobes and facultative organisms is promoted. The oxygen level depends on two basic factors: location and time. Along the root canal space, in an apical direction, oxygen tension decreases gradually. In relation to time, the

longer an infected pulp necrosis stands in a tooth non-exposed to the oral environment the more oxygen is consumed, thus giving anaerobes better conditions to survive and multiply (78, 130).

While likely to increase upon root canal treatment, the oxygen levels may still be low in the apical region. Thus bacteria escaping instrumentation and intra-canal medication in such locations will become minimally exposed to oxygen. This means that not only will facultative organisms remain viable but also species like *Fusobacterium* spp. and *Peptostreptococcus* spp. are favored, which otherwise have an extremely low tolerance to oxygen (131–133).

Bacterial interactions

Bacterial synergy is crucial for bacterial adaptation to environmental stress (26, 134). In root canals, prevailing organisms may avoid the lethal effects of chemo-

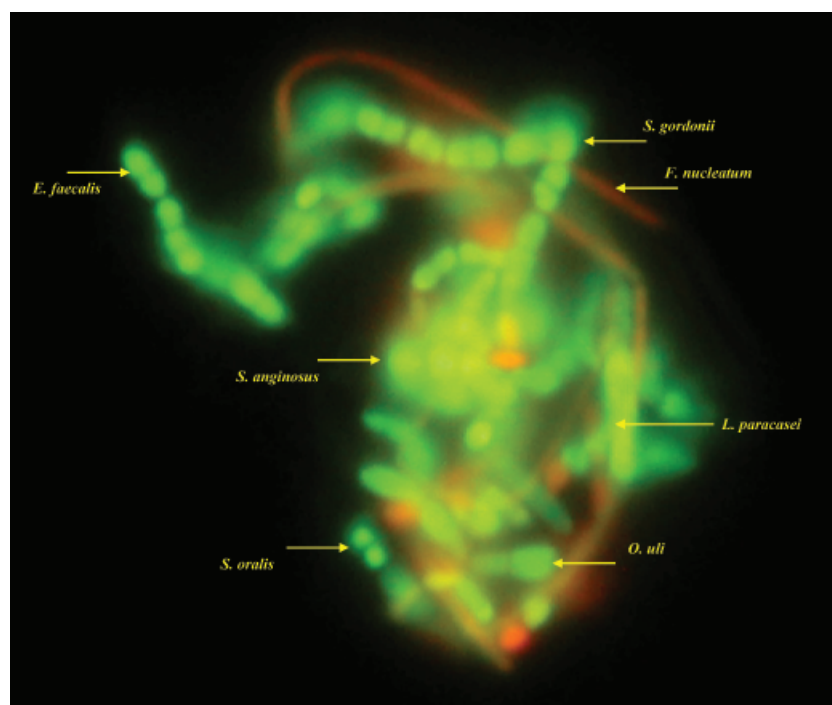


Fig. 3. Fluorescent staining of a community of seven root canal isolates grown *in vitro* under planktonic conditions and exposed to an alkaline challenge (pH 10.5) for 4h. Co-aggregation developed between *Enterococcus faecalis*, *Fusobacterium nucleatum*, *Lactobacillus paracasei*, *Olsenella uli*, *Streptococcus anginosus*, *S. gordonii*, and *S. oralis*. Green cells correspond to viable organisms while fluorescent red indicate membrane damage.

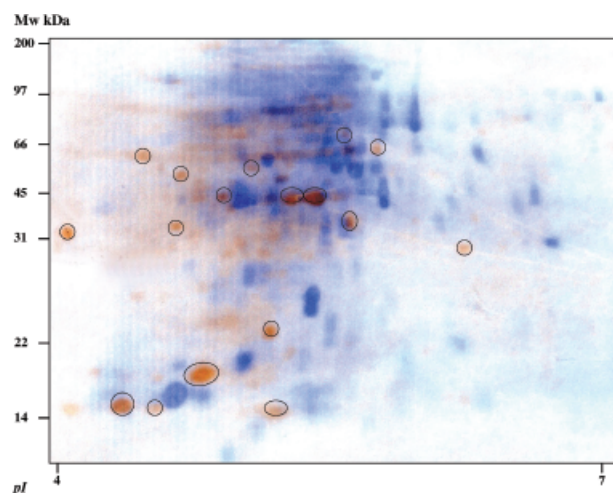


Fig. 4. Over imposed 2-D electrophoresis gels of whole-cell protein extracts (spots in blue) and extracellular proteins (spots in brown and encircled) produced by a clinical isolate of *S. oralis*. Vertical axes represent molecular weight (Mw) in kDa, the horizontal axes represent pI values of separated proteins. Produced from the data in the study by Chávez de Paz et al. (30).

mechanical preparation and intra-canal medication by adhering to available surfaces forming biofilms (see the paper by Svensäter and Bergenholtz in this issue (135).

Another important process is co-aggregation where genetically distinct bacteria in a planktonic state become attached to each other via certain molecules (136, 137). These clusters of co-aggregated bacteria will recognize and co-adhere to surface-attached cells (138). While there is no evidence that such processes occur in infected root canals, odds-ratio calculations have shown certain combinations of root canal organisms (31, 130, 139, 140). Hence, in teeth with untreated necrotic pulps, positive associations have been observed between *P. intermedia* and *P. anaerobius*, *P. intermedia* and *P. micros*, and *P. anaerobius* and *E. lentum* (130). Gomes et al. (139) and Peters et al. (140) have reported similar associations. On searching for positive associations among residual organisms after root canal treatment, little data exist. *Prevotella* spp. and *L. crispatus*, *Prevotella* spp. and *B. dentium*, and *Fusobacterium* spp. and *L. paracasei* were frequently associated in the study by Chávez de Paz et al. (31).

Stress-related proteins

Bacteria exposed to unfavorable environmental conditions respond by expressing a varied set of proteins

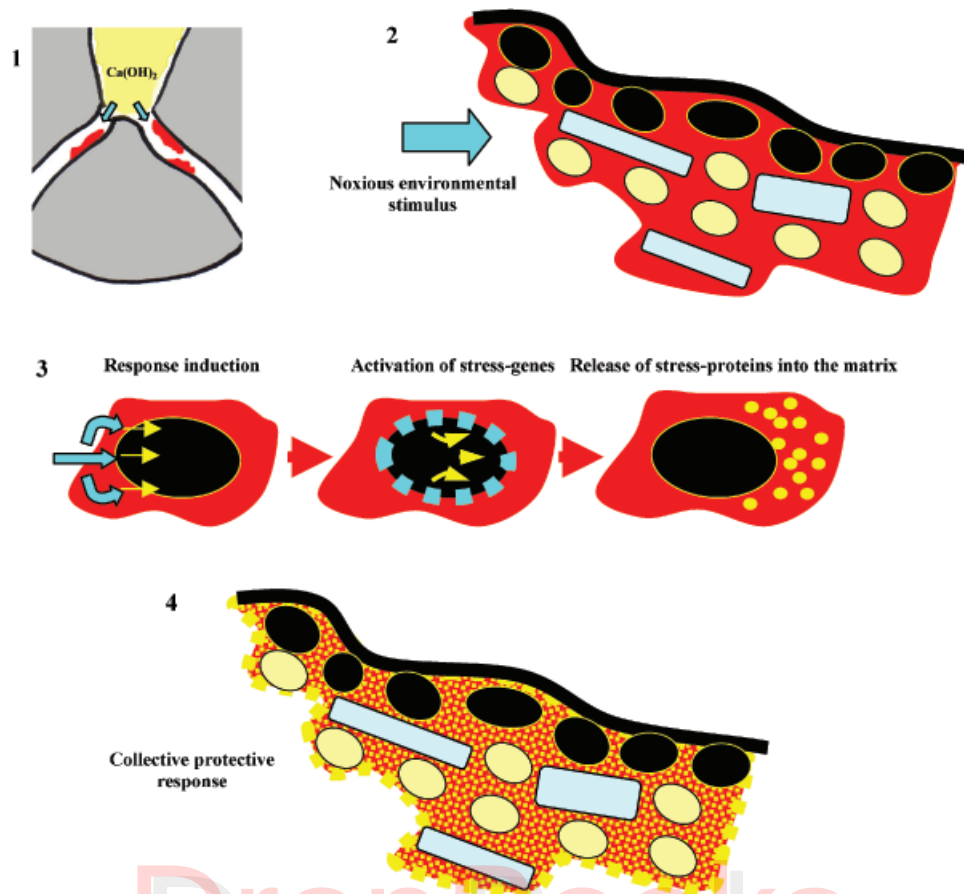


Fig. 5. Hypothetical outline of a stress protein response in biofilm communities of root canal bacteria.

(so-called stress proteins) (141). Stress proteins protect the bacterial cell against injurious factors and enhance bacterial survival. Stress proteins are multi-functional, some of which assist translocation and refolding of damaged proteins in the cell (molecular chaperones) (142). These proteins also fulfill other beneficial functions such as modulation of protein synthesis, regulation of kinases, associations with enzymes (possibly pathogenic), and participation in signal transduction pathways.

The majority of stress proteins monitor regular physiological functions. Some of these proteins are released and activated extra-cellularly under normal and stressful circumstances (143) (see also Fig. 4).

The effect on host tissue of bacterial stress proteins is not well established. In oral infections, these proteins have been studied primarily in conjunction with periodontal disease (144). In diseased periodontal sites, levels of stress proteins from species like *P. gingivalis* and *A. actinomycetemcomitans* have been found to increase (145) and to produce immune

reactions (146, 147). In endodontic infections, the pathogenic role of stress-related proteins is unknown. Possibly bacteria that are located close to the periapical foramen may release such proteins to induce inflammatory reactions in the periapical tissues.

Figure 5 illustrates a hypothetical situation where bacteria within a biofilm-like structure are thought to respond to an environmental stressor. The effect of the stressor includes response induction and activation of genes that encode stress proteins. The responses of each bacterial cell sum up and provide protective effects of benefit for the entire microbial community. Research in this area may provide important information on the adaptive capabilities of root canal bacteria to external adverse influences.

Concluding remark

The potential significance of a variety of Gram-positive organisms in endodontic infections has been highlighted in this review. While Gram-negative anaerobes

predominate in primary root canal infections, Gram-positive facultatives tend to become dominating in failing post-treatment cases. Certain genera and even species seem to persist to a greater extent than other. The mechanism behind this selection process should be explored possibly by studying stress responses and other adaptive mechanisms. Research should also elucidate the pathogenic potential of the various organisms isolated in post-treatment endodontic disease.

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References

1. Appleton JLT. Bacteriological control of the treatment of periapical infection. *Dent Items Interest* 1927; **49**: 589–597.
2. Brown LR Jr., Rudolph CE Jr. Isolation and identification of microorganisms from unexposed canals of pulp-involved teeth. *J Oral Surg* 1957; **10**: 1094–1099.
3. Crawford JJ, Shankle RJ. Application of newer methods to study the importance of root canal and oral microbiota in endodontics. *Oral Surg Oral Med Oral Pathol* 1961; **14**: 1109–1123.
4. Möller ÅJR. Microbiological examination of root canals and periapical tissues of human teeth. Methodological studies. *Odontol Tidskr* 1966; **74**(Suppl): 1–380.
5. Bergenholtz G. Micro-organisms from necrotic pulp of traumatized teeth. *Odontol Revy* 1974; **25**: 347–358.
6. Kantz WE, Henry CA. Isolation and classification of anaerobic bacteria from intact pulp chambers of non-vital teeth in man. *Arch Oral Biol* 1974; **19**: 91–96.
7. Wittgow WC Jr., Sabiston CB Jr. Microorganisms from pulpal chambers of intact teeth with necrotic pulps. *J Endod* 1975; **1**: 168–171.
8. Sundqvist G. *Bacteriological Studies of Necrotic Dental Pulps. Odontological Dissertations*. Umeå, Sweden: Umeå University, 1976.
9. Baumgartner JC, Falkler WA Jr. Bacteria in the apical 5 mm of infected root canals. *J Endod* 1991; **17**: 380–383.
10. Wasfy MO, McMahon KT, Minah GE, Falkler WA Jr. Microbiological evaluation of periapical infections in Egypt. *Oral Microbiol Immunol* 1992; **7**: 100–105.
11. Haapasalo M. *Bacteroides* spp. in dental root canal infections. *Endod Dent Traumatol* 1989; **5**: 1–10.
12. Baumgartner JC, Watkins BJ, Bae KS, Xia T. Association of black-pigmented bacteria with endodontic infections. *J Endod* 1999; **25**: 413–415.
13. Sundqvist GK, Eckerbom MI, Larsson AP, Sjögren UT. Capacity of anaerobic bacteria from necrotic dental pulps to induce purulent infections. *Infect Immun* 1979; **25**: 685–693.
14. Dahlén G, Bergenholtz G. Endotoxic activity in teeth with necrotic pulps. *J Dent Res* 1980; **59**: 1033–1040.
15. Sundqvist G, Carlsson J. Lactobacilli of infected dental root canals. *Odontol Revy* 1974; **25**: 233–238.
16. Méjare B. On the facultative anaerobic streptococci in infected dental root canals in man. *Odontol Revy* 1975; **33**(Suppl): 1–26.
17. Molander A, Reit C, Dahlén G, Kvist T. Microbiological status of root-filled teeth with apical periodontitis. *Int Endod J* 1998; **31**: 1–7.
18. Sundqvist G, Figdor D, Persson S, Sjögren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1998; **85**: 86–93.
19. Peculienė V, Balciuniene I, Eriksen HM, Haapasalo M. Isolation of *Enterococcus faecalis* in previously root-filled canals in a lithuanian population. *J Endod* 2000; **26**: 593–595.
20. Hancock HH III, Sigurdsson A, Trope M, Moisewitsch J. Bacteria isolated after unsuccessful endodontic treatment in a North American population. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2001; **91**: 579–586.
21. Pinheiro ET, Gomes BP, Ferraz CC, Sousa EL, Teixeira FB, Souza-Filho FJ. Microorganisms from canals of root-filled teeth with periapical lesions. *Int Endod J* 2003; **36**: 1–11.
22. Byström A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. *Scand J Dent Res* 1981; **89**: 321–328.
23. Gomes BP, Lilley JD, Drucker DB. Variations in the susceptibilities of components of the endodontic microflora to biomechanical procedures. *Int Endod J* 1996; **29**: 235–241.
24. Peters LB, van Winkelhoff AJ, Buijs JF, Wesselink PR. Effects of instrumentation, irrigation and dressing with calcium hydroxide on infection in pulpless teeth with periapical bone lesions. *Int Endod J* 2002; **35**: 13–21.
25. Chávez De Paz LE, Dahlén G, Molander A, Möller ÅJR, Bergenholtz G. Bacteria recovered from teeth with apical periodontitis after antimicrobial endodontic treatment. *Int Endod J* 2003; **36**: 500–508.
26. Kassen R, Rainey PB. The ecology and genetics of microbial diversity. *Annu Rev Microbiol* 2004; **58**: 207–231.
27. Love R. Invasion of dentinal tubules by root canal bacteria. *Endod Topics* 2004; **9**: 52–65.
28. Edwardsson S. Bacteriological studies on deep areas of carious dentine. *Odontol Revy* 1974; **32**(Suppl): 1–143.
29. Hoshino E, Ando N, Sato M, Kota K. Bacterial invasion of non-exposed dental pulp. *Int Endod J* 1992; **25**: 2–5.

30. Chávez de Paz LE, Svensäter G, Dahlén G, Bergenholtz G. Streptococci from root canals in teeth with apical periodontitis receiving endodontic treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, in press.
31. Chávez de Paz LE, Molander A, Dahlén G. Gram-positive rods prevailing in teeth with apical periodontitis undergoing root canal treatment. *Int Endod J* 2004; **37**: 579–587.
32. Chung WO, Demuth DR, Lamont RJ. Identification of a *Porphyromonas gingivalis* receptor for the *Streptococcus gordonii* SspB protein. *Infect Immun* 2000; **68**: 6758–6762.
33. Nonaka E, Kiyama-Kishikawa M, Hayakawa M. Identification of 40-kDa outer membrane protein as an aggregation factor of *Porphyromonas gingivalis* to streptococcus gordonii. *J Oral Sci* 2001; **43**: 239–243.
34. Lamont RJ, El-Sabaeny A, Park Y, Cook GS, Costerton JW, Demuth DR. Role of the *Streptococcus gordonii* SspB protein in the development of *Porphyromonas gingivalis* biofilms on streptococcal substrates. *Microbiology* 2002; **148**: 1627–1636.
35. Love RM, McMillan MD, Park Y, Jenkinson HF. Coinvasion of dentinal tubules by *Porphyromonas gingivalis* and *Streptococcus gordonii* depends upon binding specificity of streptococcal antigen I/II adhesin. *Infect Immun* 2000; **68**: 1359–1365.
36. Cook GS, Costerton JW, Lamont RJ. Biofilm formation by *Porphyromonas gingivalis* and *Streptococcus gordonii*. *J Periodontal Res* 1998; **33**: 323–327.
37. El-Sabaeny A, Demuth DR, Park Y, Lamont RJ. Environmental conditions modulate the expression of the sspA and sspB genes in *Streptococcus gordonii*. *Microb Pathog* 2000; **29**: 101–113.
38. Shinzato T, Saito A. The *Streptococcus milleri* group as a cause of pulmonary infections. *Clin Infect Dis* 1995; **21**(Suppl 3): S238–S243.
39. Sasaki H, Ishizuka T, Muto M, Nezu M, Nakanishi Y, Inagaki Y, Watanabe H, Terada M. Presence of *Streptococcus anginosus* DNA in esophageal cancer, dysplasia of esophagus, and gastric cancer. *Cancer Res* 1998; **58**: 2991–2995.
40. Allen BL, Katz B, Hook M. *Streptococcus anginosus* adheres to vascular endothelium basement membrane and purified extracellular matrix proteins. *Microb Pathog* 2002; **32**: 191–204.
41. Morita E, Narikiyo M, Yano A, Nishimura E, Igaki H, Sasaki H, Terada M, Hamada N, Kawabe R. Different frequencies of streptococcus anginosus infection in oral cancer and esophageal cancer. *Cancer Sci* 2003; **94**: 492–496.
42. Narikiyo M, Tanabe C, Yamada Y, Igaki H, Tachimori Y, Kato H, Muto M, Montesano R, Sakamoto H, Nakajima Y, Sasaki H. Frequent and preferential infection of *Treponema denticola*, *Streptococcus mitis*, and *Streptococcus anginosus* in esophageal cancers. *Cancer Sci* 2004; **95**: 569–574.
43. Frandsen EV, Pedrazzoli V, Kilian M. Ecology of viridans streptococci in the oral cavity and pharynx. *Oral Microbiol Immunol* 1991; **6**: 129–133.
44. Whaley RA, Beighton D. Current classification of the oral streptococci. *Oral Microbiol Immunol* 1998; **13**: 195–216.
45. Takahashi N, Yamada T. Acid-induced acid tolerance and acidogenicity of non-mutants streptococci. *Oral Microbiol Immunol* 1999; **14**: 43–48.
46. Brailsford SR, Shah B, Simons D, Gilbert S, Clark D, Ines I, Adams S, Allison C, Beighton D. The predominant aciduric microflora of root-caries lesions. *J Dent Res* 2001; **80**: 1828–1833.
47. Wilkins JC, Beighton D, Homer KA. Effect of acidic pH on expression of surface-associated proteins of streptococcus oralis. *Appl Environ Microbiol* 2003; **69**: 5290–5296.
48. Baddour LM. Virulence factors among gram-positive bacteria in experimental endocarditis. *Infect Immun* 1994; **62**: 2143–2148.
49. Froeliger EH, Fives-Taylor P. *Streptococcus parasanguis* fimbria-associated adhesin fap1 is required for biofilm formation. *Infect Immun* 2001; **69**: 2512–2519.
50. Oetjen J, Fives-Taylor P, Froeliger EH. The divergently transcribed *Streptococcus parasanguis* virulence-associated fimA operon encoding an Mn(2+)-responsive metal transporter and pepO encoding a zinc metallo-peptidase are not coordinately regulated. *Infect Immun* 2002; **70**: 5706–5714.
51. Ahrne S, Noback S, Jeppsson B, Adlerberth I, Wold AE, Molin G. The normal *Lactobacillus* flora of healthy human rectal and oral mucosa. *J Appl Microbiol* 1998; **85**: 88–94.
52. Orrhage K, Nord CE. Bifidobacteria and lactobacilli in human health. *Drugs Exp Clin Res* 2000; **26**: 95–111.
53. Felten A, Barreau C, Bizet C, Lagrange PH, Philippon A. *Lactobacillus* species identification, H₂O₂ production, and antibiotic resistance and correlation with human clinical status. *J Clin Microbiol* 1999; **37**: 729–733.
54. Tamime AY. Fermented milks: a historical food with modern applications – a review. *Eur J Clin Nutr* 2002; **56**(Suppl 4): S2–S15.
55. Hamilton-Miller JM. Probiotics and prebiotics in the elderly. *Postgrad Med J* 2004; **80**: 447–451.
56. Servin AL. Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. *FEMS Microbiol Rev* 2004; **28**: 405–440.
57. Nigatu A, Ahrne S, Molin G. Temperature-dependent variation in API 50 CH fermentation profiles of *Lactobacillus* species. *Curr Microbiol* 2000; **41**: 21–26.
58. Kersters K, De Ley J. Identification and grouping of bacteria by numerical analysis of their electrophoretic protein patterns. *J Gen Microbiol* 1975; **87**: 333–342.
59. Pot B, Hertel C, Ludwig W, Descheemaeker P, Kersters K, Schleifer KH. Identification and classification of *Lactobacillus acidophilus*, *L. gasseri* and *L. johnsonii* strains by SDS-PAGE and rRNA-targeted oligonucleotide probe hybridization. *J Gen Microbiol* 1993; **139**(Part 3): 513–517.

60. Schillinger U, Yousif NM, Sesar L, Franz CM. Use of group-specific and RAPD-PCR analyses for rapid differentiation of *Lactobacillus* strains from probiotic yogurts. *Curr Microbiol* 2003; **47**: 453–456.
61. Woo PC, Ng KH, Lau SK, Yip KT, Fung AM, Leung KW, Tam D, Que T, Yuen K. Usefulness of the MicroSeq 500 16S ribosomal DNA-based bacterial identification system for identification of clinically significant bacterial isolates with ambiguous biochemical profiles. *J Clin Microbiol* 2003; **41**: 1996–2001.
62. McKay GS. The histology and microbiology of acute occlusal dentine lesions in human permanent molar teeth. *Arch Oral Biol* 1976; **21**: 51–58.
63. Dewhirst FE, Paster BJ, Tzellas N, Coleman B, Downes J, Spratt DA, Wade W. Characterization of novel human oral isolates and cloned 16S rDNA sequences that fall in the family Coriobacteriaceae: description of *Olsenella* gen. nov., reclassification of *Lactobacillus uli* as *Olsenella uli* comb. nov. and description of *Olsenella profusa* sp. nov. *Int J Syst Evol Microbiol* 2001; **51**: 1797–1804.
64. Olsen I, Johnson JL, Moore LV, Moore WE. *Lactobacillus uli* sp. nov. and *Lactobacillus rimae* sp. nov. from the human gingival crevice and emended descriptions of *Lactobacillus minutus* and *Streptococcus parvulus*. *Int J Syst Bacteriol* 1991; **41**: 261–266.
65. Holzapfel WH, Haberer P, Geisen R, Bjorkroth J, Schillinger U. Taxonomy and important features of probiotic microorganisms in food and nutrition. *Am J Clin Nutr* 2001; **73**: 365S–373S.
66. Arpi M, Vancanneyt M, Swings J, Leisner JJ. Six cases of *Lactobacillus* bacteraemia: identification of organisms and antibiotic susceptibility and therapy. *Scand J Infect Dis* 2003; **35**: 404–408.
67. Sturdee S, Bainton R, Barnham M. Intravascular infection with *Lactobacillus paracasei*. *J Infect* 1998; **37**: 184–186.
68. Engström B. The significance of enterococci in root canal treatment. *Odontol Revy* 1964; **15**: 87–106.
69. Sirén EK, Haapasalo MP, Ranta K, Salmi P, Kerosuo EN. Microbiological findings and clinical treatment procedures in endodontic cases selected for microbiological investigation. *Int Endod J* 1997; **30**: 91–95.
70. Reit C, Dahlén G. Decision making analysis of endodontic treatment strategies in teeth with apical periodontitis. *Int Endod J* 1988; **21**: 291–299.
71. Ørstavik D, Haapasalo M. Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. *Endod Dent Traumatol* 1990; **6**: 142–149.
72. Molander A, Reit C, Dahlén G. Microbiological evaluation of clindamycin as a root canal dressing in teeth with apical periodontitis. *Int Endod J* 1990; **23**: 113–118.
73. Molander A, Reit C, Dahlén G. The antimicrobial effect of calcium hydroxide in root canals pretreated with 5% iodine potassium iodide. *Endod Dent Traumatol* 1999; **15**: 205–209.
74. Dahlén G, Samuelsson W, Molander A, Reit C. Identification and antimicrobial susceptibility of enterococci isolated from the root canal. *Oral Microbiol Immunol* 2000; **15**: 309–312.
75. Möller ÅJR, Fabricius L, Dahlén G, Sundqvist G, Happonen RP. Apical periodontitis development and bacterial response to endodontic treatment. Experimental root canal infections in monkeys with selected bacterial strains. *Eur J Oral Sci* 2004; **112**: 207–215.
76. Molander A, Dahlén G. Evaluation of the antibacterial potential of tetracycline or erythromycin mixed with calcium hydroxide as intracanal dressing against *Enterococcus faecalis* in vivo. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2003; **96**: 744–750.
77. Sunde PT, Olsen I, Debelian GJ, Tronstad L. Microbiota of periapical lesions refractory to endodontic therapy. *J Endod* 2002; **28**: 304–310.
78. Fabricius L. Oral bacteria and apical periodontitis. An experimental study in monkeys. Thesis, Göteborg University, Gothenburg, Sweden, 1982.
79. Fabricius L, Dahlén G, Holm SE, Möller ÅJR. Influence of combinations of oral bacteria on periapical tissues of monkeys. *Scand J Dent Res* 1982; **90**: 200–206.
80. Love RM. *Enterococcus faecalis* – a mechanism for its role in endodontic failure. *Int Endod J* 2001; **34**: 399–405.
81. Kayaoglu G, Ørstavik D. Virulence factors of *Enterococcus faecalis*: relationship to endodontic disease. *Crit Rev Oral Biol Med* 2004; **15**: 308–320.
82. Sedgley C, Clawell D. Bacterial plasmids in the oral and endodontic microflora. *Endod Topics* 2004; **9**: 37–51.
83. Basrani B, Tjäderhane L, Santos JM, Pascon E, Grad H, Lawrence HP, Friedman S. Efficacy of chlorhexidine- and calcium hydroxide-containing medicaments against *Enterococcus faecalis* in vitro. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2003; **96**: 618–624.
84. Turner SR, Love RM, Lyons KM. An in-vitro investigation of the antibacterial effect of nisin in root canals and canal wall radicular dentine. *Int Endod J* 2004; **37**: 664–671.
85. Saleh IM, Ruyter IE, Haapasalo M, Ørstavik D. Survival of *Enterococcus faecalis* in infected dentinal tubules after root canal filling with different root canal sealers in vitro. *Int Endod J* 2004; **37**: 193–198.
86. Gomes BP, Ferraz CC, Vianna ME, Berber VB, Teixeira FB, Souza-Filho FJ. In vitro antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of *Enterococcus faecalis*. *Int Endod J* 2001; **34**: 424–428.
87. Gomes BP, Souza SF, Ferraz CC, Teixeira FB, Zaia AA, Valdrighi L, Souza-Filho F. Effectiveness of 2% chlorhexidine gel and calcium hydroxide against *Enterococcus faecalis* in bovine root dentine in vitro. *Int Endod J* 2003; **36**: 267–275.
88. Shabahang S, Torabinejad M. Effect of MTAD on *Enterococcus faecalis*-contaminated root canals of extracted human teeth. *J Endod* 2003; **29**: 576–579.
89. Portenier I, Haapasalo H, Ørstavik D, Yamauchi M, Haapasalo M. Inactivation of the antibacterial activity of

- iodine potassium iodide and chlorhexidine digluconate against *Enterococcus faecalis* by dentin, dentin matrix, type-I collagen, and heat-killed microbial whole cells. *J Endod* 2002; **28**: 634–637.
90. Sukawat C, Srisuwan T. A comparison of the antimicrobial efficacy of three calcium hydroxide formulations on human dentin infected with *Enterococcus faecalis*. *J Endod* 2002; **28**: 102–104.
 91. Lui JN, Sae-Lim V, Song KP, Chen NN. In vitro antimicrobial effect of chlorhexidine-impregnated gutta percha points on *Enterococcus faecalis*. *Int Endod J* 2004; **37**: 105–113.
 92. Borssén E, Sundqvist G. Actinomyces of infected dental root canals. *Oral Surg Oral Med Oral Pathol* 1981; **51**: 643–648.
 93. Sundqvist G. Taxonomy, ecology, and pathogenicity of the root canal flora. *Oral Surg Oral Med Oral Pathol* 1994; **78**: 522–530.
 94. Nair PNR, Schroeder HE. Periapical actinomycosis. *J Endod* 1984; **10**: 567–570.
 95. Sundqvist G, Reuterving CO. Isolation of *Actinomyces israelii* from periapical lesion. *J Endod* 1980; **6**: 602–606.
 96. Happonen RP, Söderling E, Viander M, Linko-Kettunen L, Pelliniemi LJ. Immunocytochemical demonstration of *Actinomyces* species and *Arachnia propionica* in periapical infections. *J Oral Pathol* 1985; **14**: 405–413.
 97. O'Grady JF, Reade PC. Periapical actinomycosis involving *Actinomyces israelii*. *J Endod* 1988; **14**: 147–149.
 98. Kalfas S, Figdor D, Sundqvist G. A new bacterial species associated with failed endodontic treatment: identification and description of *Actinomyces radicidentis*. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2001; **92**: 208–214.
 99. Yeung MK. Molecular and genetic analyses of *Actinomyces* spp. *Crit Rev Oral Biol Med* 1999; **10**: 120–138.
 100. Clarridge JE III, Zhang Q. Genotypic diversity of clinical *Actinomyces* species: phenotype, source, and disease correlation among genospecies. *J Clin Microbiol* 2002; **40**: 3442–3448.
 101. Cummins CS, Moss CW. Fatty acid composition of *Propionibacterium propionicum* (*Arachnia propionica*). *Int J Syst Bacteriol* 1990; **40**: 307–308.
 102. Sjögren U, Happonen RP, Kahnberg KE, Sundqvist G. Survival of *Arachnia propionica* in periapical tissue. *Int Endod J* 1988; **21**: 277–282.
 103. Figdor D, Sjögren U, Sörlin S, Sundqvist G, Nair PNR. Pathogenicity of *Actinomyces israelii* and *Arachnia propionica*: experimental infection in guinea pigs and phagocytosis and intracellular killing by human polymorphonuclear leukocytes in vitro. *Oral Microbiol Immunol* 1992; **7**: 129–136.
 104. Siqueira JF Jr. Periapical actinomycosis and infection with *Propionibacterium propionicum*. *Endod Topics* 2003; **6**: 78–95.
 105. Germond JE, Mamin O, Mollet B. Species specific identification of nine human *Bifidobacterium* spp. in feces. *Syst Appl Microbiol* 2002; **25**: 536–543.
 106. Goodacre R, Hiom SJ, Cheeseman SL, Murdoch D, Weightman AJ, Wade WG. Identification and discrimination of oral asaccharolytic *Eubacterium* spp. by pyrolysis mass spectrometry and artificial neural networks. *Curr Microbiol* 1996; **32**: 77–84.
 107. Willems A, Collins MD. Phylogenetic relationships of the genera *Acetobacterium* and *Eubacterium sensu stricto* and reclassification of *Eubacterium alactolyticum* as *Pseudoramibacter alactolyticus* gen. nov., comb. nov. *Int J Syst Bacteriol* 1996; **46**: 1083–1087.
 108. Spratt DA, Weightman AJ, Wade WG. Diversity of oral asaccharolytic *Eubacterium* species in periodontitis – identification of novel phylotypes representing uncultivated taxa. *Oral Microbiol Immunol* 1999; **14**: 56–59.
 109. Bergenholtz G, Spångberg L. Controversies in endodontics. *Crit Rev Oral Biol Med* 2004; **15**: 99–114.
 110. Saunders WP, Saunders EM. Assessment of leakage in the restored pulp chamber of endodontically treated multirooted teeth. *Int Endod J* 1990; **23**: 28–33.
 111. Mjör IA, Smith MR, Ferrari M, Mannocci F. The structure of dentine in the apical region of human teeth. *Int Endod J* 2001; **34**: 346–353.
 112. Moreillon P, Majcherczyk PA. Proinflammatory activity of cell-wall constituents from gram-positive bacteria. *Scand J Infect Dis* 2003; **35**: 632–641.
 113. Berger-Bachi B. Resistance mechanisms of gram-positive bacteria. *Int J Med Microbiol* 2002; **292**: 27–35.
 114. Sutherland IW. The biofilm matrix – an immobilized but dynamic microbial environment. *Trends Microbiol* 2001; **9**: 222–227.
 115. Gordon TM, Ranly DM, Boyan BD. The effects of calcium hydroxide on bovine pulp tissue: variations in pH and calcium concentration. *J Endod* 1985; **11**: 156–160.
 116. Nerwich A, Figdor D, Messer HH. pH changes in root dentin over a 4-week period following root canal dressing with calcium hydroxide. *J Endod* 1993; **19**: 302–306.
 117. Esberard RM, Carnes DL Jr., del Rio CE. Changes in pH at the dentin surface in roots obturated with calcium hydroxide pastes. *J Endod* 1996; **22**: 402–405.
 118. Ardeshtna SM, Qualtrough AJ, Worthington HV. An in vitro comparison of pH changes in root dentine following canal dressing with calcium hydroxide points and a conventional calcium hydroxide paste. *Int Endod J* 2002; **35**: 239–244.
 119. Kroll RG. Alkalophiles. In: Edwards C, ed. *Microbiology of Extreme Environments*. Milton Keynes: Open University Press, 1990: 55–92.
 120. Flahaut S, Benachour A, Giard JC, Boutibonnes P, Auffray Y. Defense against lethal treatments and de novo protein synthesis induced by NaCl in *Enterococcus faecalis* ATCC 19433. *Arch Microbiol* 1996; **165**: 317–324.
 121. Flahaut S, Hartke A, Giard JC, Auffray Y. Alkaline stress response in *Enterococcus faecalis*: adaptation, cross-protection, and changes in protein synthesis. *Appl Environ Microbiol* 1997; **63**: 812–814.

122. Evans M, Davies JK, Sundqvist G, Figdor D. Mechanisms involved in the resistance of *Enterococcus faecalis* to calcium hydroxide. *Int Endod J* 2002; **35**: 221–228.
123. Booth IR, Kroll RG. Regulation of cytoplasmic pH (pH_i) in bacteria and its relationship to metabolism. *Biochem Soc Trans* 1983; **11**: 70–72.
124. Booth IR. Regulation of cytoplasmic pH in bacteria. *Microbiol Rev* 1985; **49**: 359–378.
125. Hall HK, Karem KL, Foster JW. Molecular responses of microbes to environmental pH stress. *Adv Microb Physiol* 1995; **37**: 229–272.
126. Sundqvist G, Figdor D. Life as an endodontic pathogen. *Endod Topics* 2003; **6**: 3–28.
127. Fridovich I. Oxygen toxicity: a radical explanation. *J Exp Biol* 1998; **201**(Part 8): 1203–1209.
128. Storz G, Imlay JA. Oxidative stress. *Curr Opin Microbiol* 1999; **2**: 188–194.
129. De Angelis M, Gobbetti M. Environmental stress responses in *Lactobacillus*: a review. *Proteomics* 2004; **4**: 106–122.
130. Sundqvist G. Associations between microbial species in dental root canal infections. *Oral Microbiol Immunol* 1992; **7**: 257–262.
131. Carlsson J, Frölander F, Sundqvist G. Oxygen tolerance of anaerobic bacteria isolated from necrotic dental pulps. *Acta Odontol Scand* 1977; **35**: 139–145.
132. Imlay JA. How oxygen damages microbes: oxygen tolerance and obligate anaerobiosis. *Adv Microb Physiol* 2002; **46**: 111–153.
133. Ludwig RA. Microaerophilic bacteria transduce energy via oxidative metabolic gearing. *Res Microbiol* 2004; **155**: 61–70.
134. Moat AG, Foster JW, Spector MP. Microbial stress responses. In: Moat AG, Foster JW, Spector MP. eds *Microbial Physiology*. New York, USA: Wiley-Liss, 2002: 582–611.
135. Svensäter G, Bergenholtz G. Biofilms in endodontic infections. *Endod Topics* 2004; **9**: 27–36.
136. Rickard AH, Gilbert P, High NJ, Kolenbrander PE, Handley PS. Bacterial coaggregation: an integral process in the development of multi-species biofilms. *Trends Microbiol* 2003; **11**: 94–100.
137. Foster JS, Kolenbrander PE. Development of a multi-species oral bacterial community in a saliva-conditioned flow cell. *Appl Environ Microbiol* 2004; **70**: 4340–4348.
138. Busscher HJ, Bos R, van der Mei HC. Initial microbial adhesion is a determinant for the strength of biofilm adhesion. *FEMS Microbiol Lett* 1995; **128**: 229–234.
139. Gomes BP, Drucker DB, Lilley JD. Positive and negative associations between bacterial species in dental root canals. *Microbios* 1994; **80**: 231–243.
140. Peters LB, Wesselink PR, van Winkelhoff AJ. Combinations of bacterial species in endodontic infections. *Int Endod J* 2002; **35**: 698–702.
141. Watson K. Microbial stress proteins. *Adv Microb Physiol* 1990; **31**: 183–223.
142. Houry WA. Chaperone-assisted protein folding in the cell cytoplasm. *Curr Protein Pept Sci* 2001; **2**: 227–244.
143. Macario AJ, Lange M, Ahring BK, De Macario EC. Stress genes and proteins in the archaea. *Microbiol Mol Biol Rev* 1999; **63**: 923–967.
144. Goulhen F, Grenier D, Mayrand D. Oral microbial heat-shock proteins and their potential contributions to infections. *Crit Rev Oral Biol Med* 2003; **14**: 399–412.
145. Ando T, Kato T, Ishihara K, Ogiuchi H, Okuda K. Heat shock proteins in the human periodontal disease process. *Microbiol Immunol* 1995; **39**: 321–327.
146. Tabeta K, Yamazaki K, Hotokezaka H, Yoshie H, Hara K. Elevated humoral immune response to heat shock protein 60 (hsp60) family in periodontitis patients. *Clin Exp Immunol* 2000; **120**: 285–293.
147. Tabeta K, Yoshie H, Yamazaki K. Characterization of serum antibody to *Actinobacillus actinomycetemcomitans* GroEL-like protein in periodontitis patients and healthy subjects. *Oral Microbiol Immunol* 2001; **16**: 290–295.
148. Byström A, Sundqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. *Int Endod J* 1985; **18**: 35–40.